# UPR in Cancer Development and Progression

**June 5-6, 2014**
**Rockville, Maryland**

A DCB sponsored workshop *UPR in Cancer Development and Progression* was held June 5-6, 2014 in Rockville, Maryland. The workshop was organized by Konstantin Salnikow (NCI, Division of Cancer Biology) and was co-chaired by Costas Koumenis (University of Pennsylvania) and Amy Lee (University of Southern California). The workshop brought together experts in the areas of basic biology of the UPR, the ER stress and cancer biology. This was *second* NCI sponsored workshop on "UPR in Cancer Development and Progression". The *first* NCI workshop on this topic was held in October of 2005 in Bethesda, Maryland.

Aims of the workshop included:

I. Understanding basis for abnormal protein folding and ER stress in cancer
II. Understanding the mechanisms of adaptive UPR and its role in tumorigenesis
III. Mechanisms of ER stress caused by oncogene activation
IV. Molecular components of UPR system as potential prognostic markers and therapeutic targets
V. Novel in vitro tools and animal models to study and image the UPR

## Introduction

The Unfolded Protein Response (UPR) encompasses a trio of cellular signaling cascades, PERK, IRE1/XBP1 and ATF6, activated by the accumulation of mis-folded proteins in the endoplasmic reticulum (ER). During carcinogenesis, both higher glycolytic activity and higher proliferation rate of cancer cells, which will require increased activities of ER protein folding, assembly and transport, may induce ER stress and adaptive UPR. The adaptive response occurring at the initial phase of the UPR aims to rebalance protein-folding homeostasis. If cells fail to recover from ER stress, the UPR represses the adaptive response and triggers apoptosis. Cancer cells selected for survival in constantly changed tumor microenvironment are also selected for elevated adaptive UPR. Indeed, it was shown that adaptive UPR is up-regulated in cancer cells and that contributes to cell survival under hypoxic conditions, nutrient deprivation and inflammation/oxidative stress. More recently it was shown that cell-intrinsic stresses caused by oncogenic activation of Myc, Ras and BRAF also up-regulate UPR pathways leading to increased apoptotic threshold. Moreover, chemotherapeutic agents have been shown to activate the UPR and downstream pro-survival pathways such as autophagy. More recently the induction of GRP78 has emerged as another pro-survival pathway. GRP78 a multifunctional protein, which is also a key regulator of the UPR sensors. Genetic knockdown of GRP78 blocks tumorigenesis in multiple models, validating its potential as an anti-cancer target. Agents against UPR components are currently in active development and show much promise.

## Summary of Workshop Presentations

### Session 1: UPR Biology, Signaling, Metabolism

**Gökhan S. Hotamisligil** (Harvard University)
*Endoplasmic Reticulum Function and Metabolic Homeostasis*

The endoplasmic reticulum is the main site for protein and lipid synthesis, trafficking, and the storage of cellular calcium and plays a significant role in adaptation to metabolic fluctuations. Therefore, the ER

needs to be dynamically regulated in order to accommodate the functional needs of individual cells. This dynamic is disrupted in animal models and humans with obesity and diabetes. Compromising certain aspects of ER function predisposes to metabolic disease and conversely, alleviation of ER stress or restoration of the ER adaptive folding responses by genetic or chemical means improves metabolic homeostasis in both humans and in experimental models. Understanding of the compositional and functional regulation of the ER and the mechanisms that give rise to its dysfunction in obesity and diabetes remain limited beyond the canonical UPR and cholesterol metabolism. We have taken a systematic approach to study how the different components of ER (lipids, proteins and calcium) act together to accommodate physiological dynamics and nutritional fluctuations in vivo. Through the use of lipomic, proteomic, translatomic, and transcriptomic platforms as well as comparative polysome profiling in organelle fractions and genome wide genetic screens, we are trying to establish the ER landscape that is relevant to metabolism. We hope to harness this information to develop tools for intervention and translational opportunities. In addition to endogenous ER components controlling ER function, we are also investigating the bi-directional interaction between the chronic inflammatory milieu of obesity and ER function in type 1 and type 2 diabetes. These studies have identified novel, integrated mechanisms of ER regulation in response to its physiological environment, provided insights into the metabolic functions of this organelle, and revealed mechanisms giving rise to ER stress and defective UPR in obesity. Potential intersection points between nutrients, organelle dysfunction, and metabolic control and novel translational and therapeutic opportunities emerging from these platforms that are applicable to human metabolic diseases will be discussed.

**Randal J. Kaufman** (Sanford-Burnham Medical Research Institute)
*Is Protein Mis-folding in the Endoplasmic Reticulum Oncogenic?*

The ER is a cellular compartment specialized for chaperone-assisted folding and post-translational modification of polypeptides. Protein folding in the ER requires energy and is unique due to the oxidizing environment that promotes disulfide bond formation. Disulfide bond formation is coupled with production of reactive oxygen species, i.e., oxidative stress. Disruption of ER homeostasis through increased biosynthetic load, nutrient deprivation, altered redox status, decreased calcium stores or energy depletion leads to accumulation of mis-folded protein and activation of the adaptive UPR. Unresolved protein mis-folding activates a maladaptive UPR leading to cell death. However, the fate-deciding mechanisms that result in adaptation vs. death are unknown. Our data suggest the PERK/eIF2α arm of the UPR promotes cell death through increased expression of the transcription factors ATF4 and CHOP that increase protein synthesis and cause oxidative stress. Our findings suggest that oxidative stress caused by protein mis-folding in the ER may initiate tumor formation.

**Maho Niwa** (University of California, San Diego)
*A Tale of Two Distinct Mechanisms for IRE1 RNase and Implications for the Cancer Treatment*

The ER is the initial and critical cellular site for folding and maturation of secreted proteins and proteins that reside within the secretory pathway. Changes in demand for ER functional capacity in response to environmental or developmental cues are recognized and adjusted by the UPR pathway. UPR activation

is initiated by the sensing of increased demand for ER functional capacity by IRE1, an ER transmembrane receptor kinase, leading to IRE1 kinase activation, autophosphorylation, dimerization/oligomerization, and activation of IRE1's endoribonuclease (RNase) activity. Specific cleavage of the UPR intron from XBP1/HAC1 mRNA by IRE1 RNase and subsequent exon ligation produces mRNA coding an active UPR-specific transcription factor. Interestingly, recent reports have revealed that UPR activation also leads to decreased levels of ER associated mRNAs, including INSULIN mRNA in an IRE1-dependent manner. To distinguish this decrease from HAC1/XBP1 mRNA splicing, this activity has been termed Regulated IRE1 Dependent Decay (RIDD). We established a robust in vitro RIDD RNase assay using purified recombinant IRE1 expressed in baculovirus or E. coli, demonstrating directly that RIDD is carried out by IRE1 itself, as opposed to an IRE1 induced RNase. Major differences in the cleavage of the HAC1/XBP1 mRNA intron and cleavage of ER-associated mRNA include that HAC1/XBP1 mRNA cleavage occurs only at the ends of the UPR intron whereas certain RIDD substrate RNAs are cleaved at multiple sites. In addition, it is apparent that HAC1/XBP1 exon cleavage must be coordinated with tRNA ligase activity while RIDD RNA fragments should be shielded from tRNA ligase to promote their degradation and to prevent translation. These differences raise interesting mechanistic questions with regard to IRE1. Does IRE1 somehow differentiate between XBP1/HAC1 and RIDD substrate mRNAs? And if so, what is the underlying molecular mechanism of differential recognition? Alternatively, are the different fates of HAC1/XPB1 and RIDD fragments determined by their accessibility to tRNA ligase? Using both in vitro and in vivo IRE1 activity assays, we found significant differences between the IRE1 dependent cleavage of HAC1/XBP1 mRNA and RIDD mRNAs. To our surprise, RIDD RNA does not compete IRE1 engaged in the cleavage of HAC1/XBP1 RNA out, while HAC1/XBP1 RNA was an effective competitor. For IRE1 engaged in RIDD cleavage, the converse was true; this reaction was not competed by excess of HAC1 RNA. Moreover, RIDD substrate cleavage does not require IRE1 oligomerization. Our further biochemical studies based on the structure of IRE1 revealed the presence of unique activation centers for XBP1/HAC1 and RIDD RNA substrate cleavage. Our study provides a molecular basis for designing strategies to differentially activate (inhibit) one of the Ire1 RNase activities without affecting the other. The ability to independently modulate the contributions of XBP1/HAC1 mRNA splicing and RIDD may lead to new strategies for treating multiple myeloma and other cancers where IRE1 is highly activated.

**Erick Snapp** (Albert Einstein College of Medicine)
*Detecting and Responding to ER Stress: More Than Just Mis-folded Secretory Proteins*

Mis-folded secretory proteins can stress the ER by depleting protein folding machinery, which in turn impairs folding of other secretory proteins and global ER function. To protect against mis-folded protein stress, the ER maintains high levels of chaperones. The chaperones bind both unfolded and un-foldable secretory proteins and help buffer the ER from accumulating unfolded proteins. Overwhelming levels of unfolded proteins trigger activation of the UPR. To study the role of the unfolded protein burden in regulating the UPR, we developed a live cell biosensor of unfolded protein levels. With this tool, we find that not all UPR-activating stresses require mis-folded protein accumulation. More interestingly, our biosensor has revealed that some so-called chemical chaperones, which can impact UPR activity, do not necessarily decrease the unfolded secretory protein burden. We also find that other cellular stress pathways, in parallel with the UPR, play roles in restoring ER homeostasis. Together, our results suggest

the key functions of the UPR are more complex than simply decreasing unfolded secretory protein levels.

## Session 2: Protein Folding, Glycosylation, Quality Control and ERAD

**Allan M. Weissman** (Center for Cancer Research, NCI/NIH)
*Insights from Studying Ubiquitination at the Endoplasmic Reticulum*

ER-associated degradation (ERAD) represents a major means of eliminating excess, mis-folded and unassembled proteins from the secretory pathway and is up-regulated as a means of responding to ER stress and the UPR. gp78, also known as the human tumor autocrine motility factor receptor (AMFR) or RNF45, is a RING finger ubiquitin ligase (E3) resident to the ER that is implicated in the targeting for degradation of an increasing number of diverse substrates. We have been studying gp78 with regard to both its physiological functions and as a model of a multi-domain single subunit ubiquitin ligase. We identified gp78 as the first known pro-metastatic ubiquitin ligase. We have also determined that it contains a novel secondary binding site for its cognate ubiquitin-conjugating enzyme (E2), a finding that has now been expanded to other ubiquitin ligases. This likely represents a means by which the low affinity interaction of RING finger proteins with E2s results in efficient and processive ubiquitination of substrates. Findings from our laboratory regarding these functions of gp78 will be presented.

**Linda M. Hendershot** (St. Jude Children's Research Hospital)
*Assembly-dependent Folding and Localization Underlies Quality Control of Multi-protein Complexes*

The proper maturation of nascent polypeptide chains entering the ER lumen are both assisted and monitored by resident ER molecular chaperones. In the case of BiP, its substrate binding domain accommodates extended polypeptide chains and recognizes sequences of 7-9 amino acids composed primarily of aliphatic/aromatic resides. These features are hallmarks of unfolded proteins and disappear into the interior of the protein when folding is complete, thus providing the basis of chaperone-dependent quality control. However many cell surface and secreted proteins are composed of multiple subunits. Their assembly is carefully monitored by the quality control machinery, although our understanding of the molecular mechanisms underlying this critical checkpoint is less well understood. We have examined a variety of heterotypic integral membrane and secreted protein complexes and demonstrate that assembly is not dependent on the complete prior folding of individual subunits. Instead, using in vitro folding assays we find that whole domains on certain subunits remain intrinsically disordered prior to assembly, which correlates with BiP binding, lack of oxidation, and retention in the ER when individual subunits are examined in cell-based assays. We show that subunit assembly is required to initiate folding/oxidation of these domains and for transport further along the secretory pathway. We also examined several integral membrane proteins, whose assembly is known to be dependent on interactions between transmembrane (TM) regions that utilize charged residues, which compromise their hydrophobicity, to facilitate this. We show these less hydrophobic TM regions can enter the ER lumen completely where they are recognized by the Hsp70 chaperone BiP and targeted for degradation. Specific subunit interactions en route to the native receptor promote membrane integration of the less hydrophobic TM segments, stabilizing the protein. Thus, membrane integration

linked to protein assembly allows cellular quality control of membrane proteins and connects the lumenal ER chaperone machinery to membrane protein biogenesis.

**Richard D. Cummings** (Emory University School of Medicine)
*Mis-folding of the T-Synthase Due to Loss-of-Function of the Molecular Chaperone Cosmc*

The biosynthesis of complex glycoconjugates including glycoproteins and proteoglycans is essential to animal development and cellular differentiation and frequently altered in tumor cells. We discovered that a key regulatory enzyme in O-glycosylation of glycoproteins to generate the ubiquitous O-glycan precursor core 1 Galβ1-3GalNAcα1-Ser/Thr in glycoproteins is the T-synthase (core 1 β3 galactosyltransferase or C1GalT1). This enzyme is itself dependent on a novel molecular chaperone in the ER that we discovered and abbreviated Cosmc (Core 1 β3-Gal-T-specific molecular chaperone). Defects in Cosmc expression result in mis-folding of the T-synthase, its accumulation in the ER, and subsequent regulated disposal of the mis-folded T-synthase in the 26S proteasome. Cosmc recognizes a unique peptide sequence in unfolded T-synthase leading to its interaction co-translationally and thus prevents undesired oligomerization of T-synthase. Cosmc is X-linked in humans (Xq24) and in mice (Xc3). Expression of Cosmc in human and murine cancers can be compromised due to altered Cosmc expression, arising from point mutations, deletions, including loss-of-heterozygosity, and epigenetic silencing, all of which can lead to accumulation of inactive T-synthase in the ER. Murine models in which Cosmc is selectively deleted in epithelial cells lining the gastrointestinal tract lead to loss of T-synthase and many abnormalities in cellular function and transformation. Thus, functional and normal expression of the key chaperone Cosmc, which can be compromised in tumor cells, has a profound effect on cellular phenotypes and survival.

## Session 3: Novel and Unanticipated Roles of UPR in Cancer

**Junying Yuan** (Harvard Medical School)
*Chemical Biology of the UPR Pathway*

The ER, an intracellular organelle involved in folding and modification for proteins to be exported to the cell surface and extracellular space, is essential for cellular function and survival. Conditions that interfere with the normal operations of ER can lead to a reduction in the ability of protein folding and consequently, the accumulation of unfolded/mis-folded proteins. The perturbation of ER functions, a phenomenon termed "ER stress", activates a number of pathways to counteract the associated damages; these pathways are together called the UPR. The UPR has a dualistic function: its activation can help to alleviate the accumulation of mis-folded proteins and reduce ER stress; on the other hand, sustained activation of UPR can lead to cell death through apoptosis. Cancer cells have been suggested to have an altered state of the UPR and resistance to ER stress. However, the role of ER stress and the UPR in cancer is still not clear. We have been studying the regulation of ER stress using small molecules as probes. We identified G05, a small molecule that can induce autophagy and inhibit ER stress-induced apoptosis. G05 activates PERK and IRE1 branches of the unfolded protein response (UPR). Both PERK and IRE1 pathways are essential for the protective effect of G05. In response to G05, autophagy pathway is induced in a mTOR-independent but eEF2K-dependent manner. The inhibition of PERK or IRE1 pathway suppresses autophagy induction. Furthermore, G05 treatment attenuates the interaction between Bip and IRE1, which may lead to the induction the IRE1 pathway. In addition, we report GC1, a small molecule that can inhibit tunicamycin (Tm) induced ER stress related apoptosis while inducing

autophagy. GC1 is able to delay Tm induced BIP and CHOP up-regulation independently of PERK pathway. Its protective effect does not depend on the classical UPR response pathway, but requires the Hrd1-dependent endoplasmic-reticulum-associated protein degradation (ERAD) machinery. Cells treated with GC1 accumulate EDEM1, which in turn promotes the extraction of targets from Calnexin/ Calreticulin folding cycle. In addition, GC1 treatment decreases the levels of EDEM1 bound to SEL1L, which leads to its lysosomal degradation. Based on our results we hypothesized that GC1 is able to alleviate Tm-driven apoptosis by inhibiting ERAD "tuning". Taken together, these two small molecules, G05 and GC1, define novel points in the ER stress pathway that can be chemically modulation to manipulate UPR response.
Acknowledgement: This work was supported in part by a grant from the UCB Pharmaceuticals, Inc.

**Tom Rutkowski** (University of Iowa Carver College of Medicine)
*Regulation of Metabolism and Oncogenesis by Stress in the Liver: Non-canonical Pathways and Unexpected Outcomes.*

The UPR was originally identified as set of pathways that restore protein folding upon its disruption in the ER. However, the ER is deeply integrated into a number of physiological processes quite apart from its role in protein folding, suggesting that disruption of ER homeostasis will impact other cellular pathways, and that the effects of flux through these pathways might redound to ER function. Here, focusing on hepatic metabolism and tumorigenesis, I will highlight two emerging examples of UPR signaling in physiological control. First: in contrast to the genes that are up-regulated by the UPR, which largely encompass the protein synthesis, folding, and secretion machinery and are relatively consistent from cell type to cell type, the genes that are suppressed by the UPR regulate diverse physiological processes that ostensibly have little to do with ER protein folding, and these genes vary considerably from one cell type to another. We are working to identify the mechanisms by which these genes are regulated and the impacts of such regulation on inflammation and metabolism. And second: the links between ER stress and inflammation and metabolism can lead to seemingly counterintuitive roles of the UPR to disease. These roles include that of the stress-regulated transcription factor CHOP contributing to hepatocellular carcinoma despite its promotion of apoptotic cell death. The aim of this talk is to stimulate discussion on the potential broader relevance of these features of the UPR to oncogenesis.

**Alan Diehl** (University of Pennsylvania)
*The Two Faces of PERK in Tumor Initiation and Tumor Progression*

Rapidly proliferating cancer cells must thrive in a microenvironment wherein metabolic nutrients such as glucose, oxygen and growth factors become limiting as tumor volume expands beyond the established vascularity of the tissue. In normal cells, limits in nutrient availability trigger growth arrest and/or apoptosis thereby preventing cellular expansion under such conditions. The Unfolded Protein Response/UPR plays a key role in sensing limitations in glucose and oxygen availability. UPR signaling in turn functions as a key node in the ensuing cell fate decisions. PERK, one of three proximal signal transducers of the UPR plays a central role in mediating cell fate decisions. The pro-survival function of PERK has garnered it considerable interest from the point of view of developing small molecule inhibitors of its catalytic activity and the hope that such inhibitors would have potent anti-tumor activity. Indeed, in the previous funding cycle, we demonstrated that PERK inhibition is of potential

clinical benefit in metastatic breast cancer. However, because PERK also pro-apoptotic and anti-proliferative activities, it could also exhibit tumor suppressive activity. Central our ability to effectively target PERK is a complete understanding of both its anti-proliferative/pro-apoptotic as well as pro-survival functions. Using a mouse harboring a conditional PERK allele, we have investigated the contribution of PERK to melanoma initiation and progression. Characterization of BRAFV600E driven melanoma in the context of PERK deficiency provides evidence that complete loss of PERK indeed compromises melanoma initiation and compromises survival of established human melanoma derived tumor cell lines. Paradoxically, additional analysis of heterozygous PERK knockouts has revealed that PERK functions as a haplo-insufficient suppressor of melanoma initiation. These studies are providing critical new insights into the mechanisms whereby the PERK protein kinase regulates cell homeostasis in response to stress.

## Session 4: Targeting the UPR in Cancer

**Amy S. Lee** (University of Southern California)
*Critical Role of GRP78/BiP in Tumorigenesis and Targeted Cancer Therapy*

GRP78/BiP, a 78 kilodalton glucose regulated protein encoded by the human gene HSPA5, is a multi-functional protein that can impact cancer via diverse mechanisms (1, 2). While the majority of GRP78 resides in the ER lumen and plays a major role in protein processing and regulates activation of the ER stress transducers, GRP78 can be translocated to compartments outside the ER, including the cell surface, where it binds novel interacting partners and exerts new functions in cell growth, signaling and viability. A major oncogenic function of cell surface GRP78 has been attributed to the activation of the phosphoinositide (PI3K) pathway. In studies performed in cancer cell lines as well as in mouse models, GRP78 is found to regulate tumor cell proliferation, apoptosis, autophagy, angiogenesis, metastasis, inflammation and stem cell renewal. The recent discovery that GRP78 is preferably expressed on the surface of tumor cells in vivo enables specific tumor targeting with minimal harmful effects on normal cells. In preclinical studies, a novel anti-GRP78 monoclonal antibody (MAb159) modulates the PI3K pathway without compensatory MAPK pathway activation. The humanized MAb159 antibody halts tumor progression in spontaneous tumor models and inhibits human cancer growth and metastasis in xenograft models with minimal toxicity. Our results suggest targeting GRP78 holds promise as a novel approach to combat cancer progression and recurrence.

**Constantinos Koumenis** (University of Pennsylvania)
*The Role of the UPR Effector ATF4 in Regulation of Anoikis and Metastasis via Cooperative Up-regulation of Heme Oxygenase I with Nrf2*

The UPR is a conserved signaling pathway with a critical role in cancer cell survival under multiple stress conditions. Targeting UPR can result in inhibition of tumor growth and proliferation. Here we show that the activating transcription factor 4 (ATF4) – a master transcriptional effector of the UPR, acts as a pro-tumorigenic factor by protecting cells against anoikis – a form of cellular apoptosis in response to matrix detachment for subsequent tumor metastasis. We demonstrate that ATF4 activates a coordinated program of cytoprotective autophagy and anti-oxidant response, primarily by transcriptionally up-regulating expression of heme oxygenase 1 (HO-1), resulting in amelioration of elevated oxidative stress

following matrix detachment. Reconstituting ATF4 expression or HO-1 expression in ATF4-deficient cells, reduces anoikis and rescues tumor lung colonization in vivo. Moreover, substantially increased expression of HO-1 was found in human patient samples of primary and metastatic tumors compared to normal epithelial or stromal tissue and increased expression of HO-1 correlated with reduced overall survival in patients with lung adenocarcinoma and glioblastoma. These results establish HO-1 as a critical mediator of ATF4 in tumor metastasis and further supports the notion of UPR being an attractive therapeutic target for malignancies. The broader implications of ATF4 in the regulation of ROS levels, autophagy and metastasis will be discussed.

**Robert Clarke** (Georgetown University Medical Center)
*The UPR - a Central Sensor of Cell Stress and Driver of Cell Fate Decisions in Breast Cancer*

The UPR has been implicated in driving breast cancer progression for over a decade. For example, a series of studies in breast cancer patients have shown expression of several key components of the UPR, including spliced XBP1, in both estrogen receptor positive (ER+) tumors (often referred to as luminal breast cancers) and in those that do not express ER, progesterone receptor (PR) or the activated HER2 oncogene (usually referred to as triple negative breast cancers; TNBC). Studies in both ER+ and ER- cell models have implicated activation of the UPR in conferring resistance to endocrine and cytotoxic therapies. We will discuss the role of the UPR in breast cancer with a specific focus on ER+ breast cancers and responsiveness to endocrine therapies. We will show studies from our NCI-funded Center for Cancer Systems Biology that have used both computational and mathematical modeling to obtain novel insights into the topology of UPR signaling modules. These models, and subsequent cellular and molecular studies, will show how XBP1, GRP78, and other components of the UPR are differentially expressed/regulated in sensitive and resistant breast cancer cells and tumors. We will also show how some components of the UPR offer novel targets for drug discovery. For example, we have used *in silico* modeling to find novel compounds that block activation of XBP1 and also down regulation of GRP78. Finally, we will also show how activation of key components of the UPR provide integrated signaling to affect both mitochondrial function and cell survival, and regulation of autophagy and cellular metabolism, to determine breast cancer cell fate outcomes.in the ER may initiate tumor formation.

## Session 5: UPR as a Tumor Adaptation Mechanism: Roles in Autophagy, Immunity, Tumor Dormancy and Metastasis

**Brad Wouters** (Ontario Institute for Cancer Research)
*UPR Activation and Its Importance During Hypoxia*

Hypoxia is a potent activator of the UPR, a conserved pathway that responds to ER stress. The UPR consists of 3 primary signaling arms activated by 3 unique sensor of ER stress - PERK, IRE-1, and ATF6. We have identified a specific, oxygen-sensitive phase of disulfide bond formation within the ER, as the underlying basis for their activation during hypoxia. Using isogenic models and newly developed small molecule inhibitors, we find that the PERK signaling arm of the UPR is uniquely important both for the survival and increased metastatic capacity of hypoxic cells. PERK dependent activation of ATF4 is required for both maintenance of autophagic flux, and protection against reactive oxygen species (ROS).

ATF4 induces genes that mediate cysteine uptake and its incorporation into glutathione, which is required to mitigate against ROS produced during cyclic hypoxia. Consequently, the PERK pathway is required for the survival of a fraction of radiation resistant hypoxic cells in tumors that contribute to long-term treatment failure following therapy. Inhibition of autophagy exacerbates ER stress, and sensitizes cells to hypoxia and/or inhibition of ROS detoxification pathways controlled by PERK signaling. Together these data imply that PERK signaling is a critical contributor to both tolerance and aggressive phenotypes of therapy resistant cells that arise as a consequence of tumor hypoxia and thus a therapeutic target in curative treatments for solid cancers.

**Zihai Li** (University of South Carolina)
*Impact of UPR on the Host Immune System: Puzzles and Opportunities*

One of the primary functions of UPR is to maintain protein homeostasis in the secretory pathway, in response to increased demand for protein folding machinery in the ER. Recent evidence suggests that UPR could have a far-reaching field-effect in multi-cellular organisms that it can regulate inflammation and host immunity that directly impacts on health and disease. I will summarize our work on one of the understudied UPR effector molecules, grp94 (gp96). Some of the key insights into grp94 biology have emerged recently including: (1) grp94 is a master chaperone for Toll-like receptors and integrins; (2) it is required for both early T and B cell development; (3) its expression can dictate inflammation vs tolerance with a disastrous consequence upon dysregulation; (4) it is an obligatory chaperone for folding platelet glycoprotein receptor complex, GPIb-IX-V, and is thus required for normal hemostasis; (5) more recently, it is found to be indispensable for folding Wnt co-receptor LRP5/6; and (6) it controls the function of tumor associated macrophages and plays key roles in inflammation-associated colon cancer. Given that all arms of UPR can tune grp94 expression, these findings suggest that UPR can regulate host immunity in more profound ways than we have expected. Questions that the UPR field must answer include: (1) is protein mis-folding the universal cue to turn on host immune defense in diseases such as cancer and aging? (2) is UPR dysregulation the driver or bystanding force for human diseases? (3) how can UPR pathway be manipulated in order to achieve immune fitness and therapeutic benefit against diseases such as cancer? (4) why all three biological processes (UPR, inflammation and cancer) converge onto a single ER protein grp94 (gp96)?

**Zeev Ronai** (The Sanford-Burnham Medical Research Institute)
*Fine Tuning of the UPR by the Ubiquitin Ligase Siah1/2*

Although work in recent years has shed light on the effects of hypoxia and ER stress on cancer cell biology, it is not yet clear how these pathways may be linked. Our preliminary results provide new insight into the role played by the ubiquitin ligases Siah1 and Siah2 as mechanistic link between ER stress and hypoxia. Siah1/2 control steady-state levels of proteins by targeting them for proteasomal degradation. Our work over the past decade has established the importance of Siah2 in controlling the availability of the hypoxia-induced transcription factor HIF1α, and its implications for melanoma and prostate cancer. More recent studies have identified the transcription factors ATF4 and sXBP1, two key ER stress response factors, as primary regulators of Siah1/2 transcription. Siah2 is not only induced by ER stress but also contributes to the magnitude of the ER stress response by controlling the availability

of ATF4. Siah2 forms a link between ER stress and hypoxia through its regulation of the prolyl hydroxylases PHD1 and PHD3, two key negative regulators of HIF1α and ATF4. Among the physiologically-relevant ER-stress conditions are oxygen glucose deprivation – mimicking ischemia, a condition in which there is insufficient blood flow to meet oxygen and metabolic demands, is commonly seen in solid tumors and is a pathophysiological representation of hypoxia and ER stress. Our ongoing studies aim at testing the hypothesis that Siah ubiquitin ligases play central roles in tumor development, progression, and response to therapy by regulating the cellular response to hypoxia and ER stress.

## Outcomes and Unresolved Issues

During two open discussion sessions, at the end of the first and second days of the workshop participants summarized emerging questions and ideas in the field of UPR biology and its role in cancer. The main outcomes of these discussion sessions are summarized below. Workshop participants who participated in the first NCI workshop on UPR biology in cancer felt that although significant progress has been made in the past nine years some questions still remain unanswered.

### Open questions

- What is the role of $Ca^{2+}$ imbalance in UPR, and which comes first? Does $Ca^{2+}$ affect protein translocation to membrane?
- Do epigenetics affect UPR signaling in cancer?
- What is the role of inflammation in UPR? Does inflammation have an "amplifying effect"?
- What is the relation between UPR and autophagy-mitophagy?
- How does ER glycosylation impact O-mannosylation and N-glycosylation of proteins and how does this impact protein folding and retrotranslocation?
- How do we dissect cis-vs –trans effects of UPR within the tumor microenvironment?
- How much of PERK function is truly dependent upon catalytic activity of eIF2a-P?

### Prediction and validating ER stress and UPR

- Is there "stress free" ER?
- Can we define "oncogenic UPR"?
- What determines selective activation of one UPR arm over others; is this activation stress specific?
- Is up-regulation of IRE1 levels a measure of UPR activation and if not, can the cancer field put out criteria paper for demonstrating a link to the UPR?

### UPR signatures

- Do the same principles of UPR & cancer links apply universally to all cell types or is this going to be highly cell/tumor type specific?
- Is it feasible to develop a comprehensive set of markers for the different UPR pathways and apply to monitor tumor initiation, progression and metastasis?

### UPR and cancer therapeutics and diagnostics

- What is the impact of protein folding/secretion load on resistance or sensitivity to drugs?
- What is the involvement of ERAD in cancer development and can we manipulate ERAD as a therapeutic opportunity?

- Does cancer therapy-induced UPR generate phenotypic diversity, which the clonal evolution selects for drug resistance?
- What is the significance of “fine-tuning” UPR for tumor development and therapy?
- Can the timing and thresholds of UPR be identified in cancer types (different malignancies) and exploited as a means to maintain dormancy (growth arrest)?